To: Len Ashack
From: Barbara McDowell
Subject: Anderson Wastewater Treatment Facility *E. coli* study
Date: January 5, 2001

During the 2000 disinfection season, the Anderson Wastewater Treatment Facility conducted a comparison study of two methods for the enumeration and detection of *Escherichia coli (E. coli)*: The U.S. EPA recommended m-TEC MF method and Colilert® using Quantitray® from IDEXX. Fifty water samples were split and tested.

The NPDES permit for the Anderson facility is in the process of renewal. Under the terms of the existing permit, the facility is not required to enumerate and report *E. coli* as a parameter. However, with the pending renewal and new permit requirements, *E. coli* limits will become a part of the renewal document.

In order to obtain permission from IDEM to use a method other than the recommended method (1103.1 MF method using m-TEC agar) for the detection and enumeration of *E. coli,* Anderson Wastewater Treatment Facility was advised to submit a document detailing a study comparison between the recommended method and the method using Colilert® that contained the following:

- Study should include 50 split samples
- Statistical analysis of the study that would show a relationship between the two methods and the degree of confidence that can be stated about the relationships
- Explanation of the relationship between the enumeration criteria for the two methods
- Verification of a portion of the bacteria identified as *E. coli* and false positive and false negative percentages for each method

Comments about the report:

1. **Study contains an adequate number of samples.**
2. **Samples were properly prepared, processed and incubated.**
3. **Proper quality control was observed.**
4. **Correct statistical analyses are included. ***
5. **Contains an explanation about the relationship between CFU and MPN. ****
6. **A random portion of samples (plates and trays) was verified. *****

*Correct statistical analysis:

Two statistical analyses were conducted at the conclusion of this study: the *Student's t Test for Correlated Samples* and a Regression Analysis.

The *Student's t Test for Correlated Samples* can be used effectively to determine if

there are significant differences in the means of two populations with both independent and dependent sampling. For our purposes, we were interested in dependent sampling, (i.e. sampling with the same population before and after sampling). **The result from this test shows that there is no evidence of significant difference between the means of the enumeration results of the two methods. This result is at a 95% confidence interval.**

The regression analysis can be used to show the relationship between interval scaled variables with **the "r^2" value defined as the "variation in "Y" explained by "X" and bounded by "0" and "1"**.   With "1" equaling a close relationship and "0" equaling no relationship. **In this case, the "r^2" value is "0.97", meaning that there exists a close relationship between the results of the sampling.**

**<u>Contains an explanation about the relationship between CFU and MPN</u>:

The Colilert® product, though not a membrane filtration method, can be reported using CFU enumeration by utilizing the chart that is part of the product package. The statistically close relationship between the two methods as noted above verifies the accuracy of the chart calculations.

*** <u>**A random portion of samples (plates and trays) was verified**</u>:

The false positive rate for the m-TEC MF method was 9.4%, which is close to the expected average of 9%. This rate was obtained through the verification process.

The false positive rate for the Colilert® method was 30.6%, which, though seemingly extraordinarily high, can be explained as follows: The Colilert® product, being a well of liquid, does not lend itself to the verification procedure used in this study. Withdrawing a loop of positive media from a well will not guarantee that *E. coli* will be contained in that specific loop, even though the well fluoresced. Finding the specific *E.coli* bacteria within the liquid contained in the well would be chance. Therefore, this high percentage rate should be disregarded because of the method used to obtain the bacteria.

<u>Conclusion and Recommendation</u>:

Statistical evidence shows strong evidence that the two methods produce reliably similar results. There is a 95% chance that results will fall within a similar range when comparing one method to the other.

We can allow the Anderson facility to use the Colilert method to detect and enumerate *E. coli* with 95% confidence that the results will be in a range that would be similar to the m-TEC method.

NOTE: Our announcement letter allowing Colilert to be used for *E. coli* detection states that we will allow permittees to use Method 9223 B in *Standard Methods* 20th Edition. The introduction to Method 9223 states that "laboratories planning to use this procedure should conduct parallel quantitative testing…. with one of the standard

coliform tests to assess the effectiveness of the test for the specific water type being analyzed and to determine comparability of the two techniques**". I would want to require any facility that decides to use Colilert to conduct this study and follow the guidelines as stated in *Standard Methods*.** This requirement will give us support for allowing the facility to use Colilert and increase our base of information comparing the two methods.